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(57) Abstract

The invention provides methods for enhancing cognitive activity and stimulating memory capacity, comprising the step of administering an effective amount of a compound with $GABA_c$ receptor antagonist activity to an animal in need of such treatment. Preferably the compound has selective $GABA_c$ receptor antagonist activity, and more preferably comprises a phosphinic acid group. The invention also provides novel compounds and compositions. The methods of the invention are useful in the treatment of dementias and conditions involving cognitive deficit, or memory impairment.

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NEUROLOGICALLY-ACTIVE COMPOUNDS

This invention relates to neurologically-active compounds, and to methods of use thereof. In particular the invention relates to methods of enhancing cognitive activity using compounds which are antagonists of $GABA_C$ receptors. Preferred compounds for use in the methods of the invention are TPMPA and analogues thereof, and novel compounds are disclosed.

BACKGROUND OF THE INVENTION

There are three major classes of GABA receptors in the central nervous system (CNS): $GABA_A$, $GABA_B$ and $GABA_C$ receptors. The pharmacology of $GABA_A$ and $GABA_B$ receptors has been extensively investigated, but $GABA_C$ receptors have been only recognised recently, and their pharmacological potential is still unknown (Johnston, 1996b).

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS), and activates three major subtypes of GABA receptors, the $GABA_A$, $GABA_B$ and $GABA_C$ receptors. $GABA_A$ receptors are ligand-gated Cl^- channels which are inhibited by the alkaloid bicuculline (Johnston, 1996a). These are heterooligomeric receptors made up of α, β, γ, and δ subunits. $GABA_B$ receptors are transmembrane receptors coupled to second messenger systems and Ca^{2+} and K^+ channels via G-proteins. These receptors are not blocked by bicuculline, but are activated by (-)baclofen and 3-aminopropylphosphinic acid (CGP27492) and blocked by phaclofen and saclofen (Kerr and Ong, 1995).

$GABA_C$ receptors (sometimes called $GABA_{NANB}$ or r receptors) were first proposed when a series of conformationally restricted GABA analogues, including *cis*-4-aminocrotonic acid (CACA), that had bicuculline-insensitive depression actions on neuronal activity, showed no affinity for [^{3}H]baclofen binding sites in rat

cerebellar membranes (Drew *et al*, 1984). $GABA_C$ receptors with similar pharmacology were first found in neurons from rat retina (Feigenspan *et al*, 1993) and white perch retina (Qian *et al*, 1993). In rat retina, rod bipolar cells contain bicuculline-insensitive, baclofen-insensitive receptors that were activated by CACA (Feigenspan *et al*, 1993). These were detected by the co-application of GABA with 100 μM bicuculline to abolish the $GABA_A$ component (Feigenspan *et al*, 1993). In white perch retina, rod-driven horizontal cells (H4) and not bipolar cells showed $GABA_C$ receptor-like pharmacology. Application of GABA on bipolar cells showed rapid desensitisation, while on rod-driven horizontal cells, desensitisation was not observed (Qian *et al*, 1993). Subsequently, $GABA_C$ receptors were found on cone-driven horizontal cells in catfish (Dong *et al*, 1994) and bipolar terminals in tiger salamander (Lukasiewicz *et al*, 1994).

The expression of mRNA from bovine retina in *Xenopus* oocytes showed that GABA activated two distinct GABA receptors. Both receptors activated Cl^- currents. One was mediated by $GABA_A$ receptors and was blocked by bicuculline, and the other was mediated by $GABA_C$ receptors and was insensitive to both bicuculline and baclofen (Polenzani *et al*, 1991). Subsequently, two cDNAs that have 30-38% sequence identity with $GABA_A$ receptor subunits were cloned from human retinal mRNA (Cutting *et al*, 1991; 1992). These subunits have been termed r_1 and r_2, and have 74% sequence identity (Cutting *et al*, 1991; 1992).

At least two major subtypes of $GABA_C$ receptors are now known, *namely* rho-1 and rho-2. As is known for other neurotransmitter receptor subtypes, different subtypes of $GABA_C$ receptors are likely to be involved in different aspects of nervous system function. As the rho-2 subunit is found in the hippocampus and neocortex, and these areas of the brain are important for memory, potent and selective agents for the rho-2 $GABA_C$ receptor are key compounds.

The species equivalents of the human r_1 and r_2 subunits have been cloned from rat (Enz *et al*, 1995). These show 88-99% homology with the respective human sequences. The use of PCR and *in situ* hybridisation have shown high expression of both the r_1 and r_2 subunits in rod bipolar cells. However, only the r_2 subunit is expressed in the CNS, particularly in the hippocampus and cortex (Enz *et al*, 1995). Recently, a third r subunit was cloned from rat retina cDNA (Ogurusu and Shingai, 1996). This subunit exhibits 63% and 61% sequence homology to the human r_1 and rat r_2 sequences respectively (Ogurusu and Shingai, 1996).

Expression of human r subunits in *Xenopus* oocytes generates homooligomeric GABA receptors with intrinsic Cl^- channels. These receptor ion channels are activated by GABA and CACA, but are insensitive to bicuculline, (-)baclofen, barbiturates and benzodiazepines. They have been shown to be sensitive to picrotoxin, and have been classified as $GABA_C$ receptors (Cutting *et al*, 1991; 1992; Polenzani *et al*, 1991; Shimada *et al*, 1992; Kusama *et al*, 1993a; 1993b; Wang *et al*, 1994; Bormann and Feigenspan, 1995; Johnston, 1996b).

The most potent $GABA_C$ receptor agonists known so far are *trans*-4-aminocrotonic acid (TACA, K_D = 0.6 μM) and GABA (K_D = 1.7 μM) (Woodward *et al*, 1993). TACA, a conformationally restricted analogue of GABA in an extended conformation, is also a $GABA_A$ receptor agonist (Johnston, 1996a). CACA, a conformationally-restricted analogue of GABA in a folded conformation, has moderate partial agonist activity at $GABA_C$ receptors (K_D = 74 μM), and may be the most selective agonist for this receptor subtype (Johnston, 1996b).

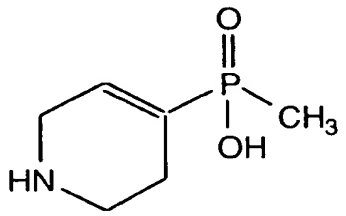
Selective agonists and antagonists are needed to determine the physiological role of $GABA_C$ receptors and to provide more specific therapeutic agents with a lower risk of unwanted side-effects. GABA is a flexible compound, due to its rotation about the C2-C3 and C3-C4 bonds. It can

exist in a range of low energy conformations (Johnston *et al*, 1978; Allan and Johnston, 1983). Two of these conformations have been restricted by the introduction of unsaturation in the form of a double bond at the C2-C3 position, and two compounds that represent these restricted conformations are CACA and TACA (Johnston *et al*, 1975). CACA and TACA have fewer degrees of rotational freedom than GABA, and can only rotate about the C3-C4 bond (Johnston *et al*, 1978; Allan and Johnston, 1983). CACA is a partially folded analogue of GABA. It has moderate activity at $GABA_C$ receptors expressed in Xenopus oocytes, and although its agonist activity is weak, it is to date the most selective agonist at these receptors, having minimal activity on $GABA_A$ and $GABA_B$ receptors (Johnston, 1996b). TACA is an extended analogue of GABA. It has potent agonist activity at $GABA_C$ receptors expressed in Xenopus oocytes; however, it is not selective, as it is also a potent $GABA_A$ receptor agonist (Johnston, 1996b).

Woodward *et al* (1993), using poly(A)$^+$ RNA from mammalian retina expressed in *Xenopus oocytes*, tested many $GABA_A$ and $GABA_B$ receptor agonists and antagonists to determine a pharmacological profile for $GABA_C$ receptors. From this study, it was found that the phosphinic and methylphosphinic analogues of GABA, which are known to be potent $GABA_B$ receptor agonists, were potent antagonists at $GABA_C$ receptors.

A series of GABA analogues was tested for agonist and antagonist activity at $GABA_C$ receptors, using poly(A)$^+$. RNA from mammalian retina injected into *Xenopus* oocytes. Several potent $GABA_C$ receptor antagonists were identified, including (3-aminopropyl)methylphosphinic acid (CGP35024; K_B = 0.8 μM), 3-aminopropylphosphinic acid (CGP27492; K_B = 1.8 μM), and 3-aminopropylphosphonic acid (3-APA, K_B = 10 μM) (Woodward *et al*, 1993). *These agents* are not selective for $GABA_C$ receptors, *as CGP35024 and CGP27492* are also very potent $GABA_B$ receptor agonists, while 3-APA is a $GABA_B$ receptor antagonist.

To date, only one specific $GABA_C$ receptor antagonist has been described. A more recently synthesised compound, 1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA), does show potent and selective $GABA_C$ receptor antagonist activity (K_D = 2.1 μM) (Murata *et al*, 1996; Ragozzino *et al*, 1996). TPMPA produces 50% inhibition of $GABA_C$ receptor activation at 2.1 μM, and has the following structure:



TPMPA

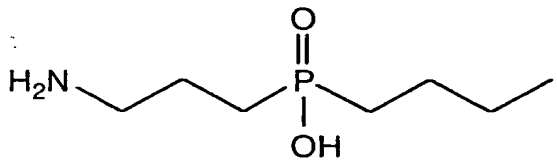
The effects of TPMPA on cognition are unknown.

As a result of the structure-activity relationship study and the selectivity of CACA for $GABA_C$ receptors, we have investigated the methylphosphinic acid and phosphinic acid analogues of CACA and the closely related analogue, TACA, as potential $GABA_C$ receptor antagonists. In this study, we demonstrate that the phosphinic and methylphosphinic acid derivatives of CACA and TACA, and 3-aminopropyl-n-butyl-phosphinic acid (CGP36742), an orally-active $GABA_B$ receptor antagonist, are $GABA_C$ receptor antagonists, and we have linked $GABA_C$ receptors with cognitive function. Extensive structure-activity studies were carried out on recombinant $GABA_C$ receptors from human retina expressed in frog oocytes. Among the compounds studied were a variety of compounds known to interact with $GABA_B$ receptors, provided by Ciba-Geigy AG, Basle.

The most interesting of the Ciba-Geigy compounds were a series of $GABA_B$ receptor antagonists that had been investigated in various memory and learning tests in rats and mice. Only one compound of the series reversed age-

related deficits of old rats (Froestl, 1995b). The cognition-enhancing effects of this compound were confirmed in learning experiments in monkeys. This compound had good oral bioavailability in rats and dogs, and in healthy young and elderly male volunteers. On this basis it was selected as a development compound for the treatment of cognition deficits.

The cognition-enhancing compound, (3-aminopropyl)-*n*-butylphosphinic acid, code- named CGP36742, has the following structure:



CGP36742

The $GABA_B$ antagonist properties of CGP36742 do not satisfactorily explain its cognition-enhancing properties, since much more potent $GABA_B$ antagonists have been described that lack these properties.

We have now surprisingly found that CGP36742 has similar potency as a $GABA_C$ antagonist to its potency as a $GABA_B$ antagonist (50% inhibition of receptor activation being found at 38 µM and 62 µM against $GABA_B$ and $GABA_C$ receptors respectively). None of the other potent $GABA_B$ antagonists showed activity against $GABA_C$ receptors. These findings indicate a likely role for $GABA_C$ receptor antagonism in the cognition-enhancing properties of CGP36742.

SUMMARY OF THE INVENTION

In one aspect the invention provides a method of enhancing the cognitive activity of an animal in need of such treatment, comprising the step of administering an effective amount of a compound which has $GABA_C$ receptor antagonist activity to said animal.

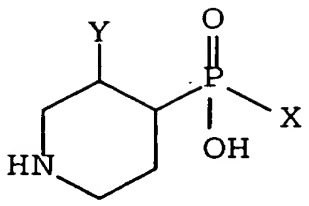
In a second aspect the invention provides a method of stimulating memory capacity, comprising the step of administering an effective amount of a compound which has $GABA_C$ receptor antagonist activity to an animal in need of such treatment.

The methods of the invention are suitable for the treatment of a variety of cognitive deficit conditions, dementias, and memory impairment conditions, including but not limited to those associated with Alzheimer's disease, AIDS, and schizophrenia.

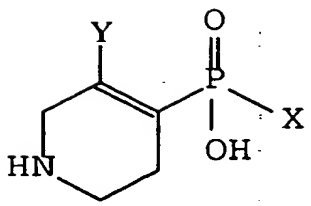
Preferably the compound has selective antagonist activity against $GABA_C$ receptors compared with $GABA_B$ receptors. More preferably, the compound has selective antagonist activity against $GABA_C$ receptors compared with $GABA_A$ receptors. Even more preferably, the compound is substantially inactive against both $GABA_A$ and $GABA_B$ receptors.

More preferably the compound comprises a phosphinic acid group, and even more preferably comprises an alkyl-substituted phosphinic acid group in which the alkyl group is of 1 to 6 carbon atoms, such as a methyl or ethyl phosphinic acid group. Most preferably the compound also comprises a double bond which imposes a conformational restriction on rotation about the bond corresponding to the C3-C4 bond of GABA. Particularly preferred compounds include, but are not limited to, conformationally-restricted analogues of CGP44530 in which rotation about the C3-C4 bond is restricted, such as TPMPA and analogues thereof.

Thus preferred compounds of the invention are represented by general formula I or general formula II,



(I)



(II)

in which X represents hydrogen, an alkyl group optionally substituted with a halogen, or a hydroxyalkyl group, and

Y represents hydrogen, a halogen, or an alkyl, alkenyl, alkynyl or acyl group, optionally substituted with halogen, nitrile, or NO_2.

In general formula I, Y may also be an alkoxy group, optionally substituted with halogen, nitrile or NO_2.

By "alkyl" is meant a straight or branched, saturated or unsaturated, substituted or unsubstituted alkyl chain of 1 to 6, preferably 1 to 4 carbon atoms, and includes alicyclic alkyl chains such as cyclopropylethyl. Alkenyl, alkynyl and acyl also refer to groups of 1-6, preferably 1-4 carbon atoms. The halogen is preferably chlorine or fluorine.

It will be clearly understood that some of the compounds which are useful for the purposes of the invention are novel, and form part of the invention. Thus in a third aspect the invention provides a compound having $GABA_C$ antagonist activity and selectivity for the rho-2 subtype of $GABA_C$ receptors of general formula II as defined above. Thus in a third aspect the invention provides a compound having $GABA_C$ antagonist activity and selectivity for the rho-2 subtype of $GABA_C$ receptors of general

formula II as defined above.

In a fourth aspect, the invention provides a composition comprising a compound of general formula II, together with a pharmaceutically-acceptable carrier.

While the invention is not in any way restricted to treatment of any particular animal species, in general the animal will be a human.

The compounds may be administered at any suitable dose and by any suitable route. Oral administration is preferred because of its greater convenience and acceptability. The effective dose will depend on the nature of the condition to be treated, and the age, weight and underlying state of health of the individual to be treated, and will be at the discretion of the attending physician or veterinarian. Suitable dosage levels may readily be determined by trial and error experimentation, using methods which are well known in the art. Similarly, suitable formulations for administration by any desired route may be prepared by standard methods, for example by reference to well-known texts such Remington: The Science and Practice of Pharmacy, Volume II, 1995 (19^{th} edition), A.R. Gennaro (Ed), Mack Publishing Company, Easton, Pennsylvania 18042, USA., or Australian Prescription Products Guide, Volume 1, 1995 (24^{th} edition), J Thomas (Ed), Australian Pharmaceutical Publishing Company Limited, Victoria, Australia.

Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", means "including but not limited to" and is not intended to exclude other additives, components, integers or steps.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows that expression of human r_1 receptors in Xenopus oocytes produces homooligomeric GABA receptors ($GABA_C$ receptors) with intrinsic Cl^- channels. GABA (1 μM) activates the Cl^- channels (duration indicated

by filled bar) and produces an inward current when the oocyte is clamped at -60 mV. (A) CGP38593 (100 μM), (B) CGP44530 (100 μM), (C) CGP70523 (100 μM), (D) CGP36742 (100 μM), and (E) CGP70522 (300 μM) do not activate the receptor (duration indicated by hatched bar). However, when (A) CGP38593 (100 μM), (B) CGP44530 (100 μM), (C) CGP70523 (100 μM), (D) CGP36742 (100 μM), and (E) CGP70522 (300 μM) are co-applied with GABA (1 μM), the GABA response is blocked or reduced.

Figure 2 shows (A) Structures of compounds that show agonist activity at $GABA_C$ receptors. (B) Structures of compounds that show antagonist activity at $GABA_C$ receptors.

Figure 3 shows structures of orally active $GABA_B$ receptor antagonists with no cognitive enhancement effects. These compounds show no affinity for $GABA_C$ receptors as either agonists or antagonists when tested at 100 μM.

Figure 4 summarizes the synthesis of PMPA by reduction fo a precursor of TMPA and subsequent hydrolysis.

Figure 5 shows the effect of TPMPA on memory formation in chicks.

Figure 6 shows the dose response relationship for the effects of TPMPA on discrimination ratio.

Figure 7 shows the effect of time after injection of TPMPA on memory formation in chicks.

Figure 8 shows the effect of TPMPA on memory for an elevated plus maze in male Swiss mice.

DETAILED DESCRIPTION OF THE INVENTION

The invention is described in detail by way of reference only to the following non-limiting general methods and experimental examples, and to the figures.

Materials

[(*E*)-3-Aminopropen-1-yl]methylphosphinic acid (CGP44530),
[(*E*)-3-aminopropen-1-yl]phosphinic acid (CGP38593),
[(*Z*)-3-aminopropen-1-yl]methylphosphinic acid (CGP70523),
[(*Z*)-3-aminopropen-1-yl]phosphinic acid (CGP70522),

3-aminopropyl-n-butyl-phosphinic acid (CGP36742), 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348), 3-aminopropyl(cyclohexylmethyl)phosphinic acid (CGP46381), (2*S*)-3-amino-2-hydroxypropyl(cyclohexylmethyl)phosphinic acid (CGP51176) and (2*R*, 1'*S*)-(3-*N*-[1'(3,4-dichlorophenyl)ethyl])amino-2-hydroxypropyl)benzylphosphinic acid (CGP55845A) were synthesised as described previously by Froestl *et al*, (1992; 1995a; 1995b). CACA and TACA were prepared as previously described (Johnston *et al*, 1975). GABA was purchased from Sigma Chemical Co (St Louis, MO, USA).

Electrophysiological Recording

Human r_1 cDNA in pcDNA (Invitrogen, San Diego, CA, USA) was obtained from Dr. George Uhl (National Institute for Drug Abuse, Baltimore, USA). The plasmid was linearized with XbaI and cRNA made using the "Message Machine" kit from Ambion Inc. (Austin, Texas, USA). 50 ng of cRNA was injected into defolliculated Stage V *Xenopus* oocytes. Two to seven days later, receptor activity was measured by two-electrode voltage clamp recording, using a Geneclamp 500 amplifier (Axon Instruments Inc., Foster City, CA., USA) and a MacLab 2e recorder (ADInstruments, Sydney, NSW, Australia). Oocytes were voltage clamped at -60 mV and continuously superfused with ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM HEPES, pH 7.5). For receptor activation measurements, the indicated concentrations of agonist and antagonist were added to ND96.

Analysis of Kinetic Data

Current (I) as a function of agonist concentration ([A]) was fitted by least squares to $I=I_{max}[A]^{nH}/(EC_{50}{}^{nH}+[A]^{nH})$, where I_{max} is the maximal current, the EC_{50} is the effective dose that activates 50% of the maximal current and n_H is the hill coefficient. EC_{50} values are expressed as mean±S.E.M. (n=3-6) and were

determined by fitting data from individual oocytes using Kaleidagraph 2.1 (1990). Current (I) as a function of antagonist concentration ([Ant]) was fitted by least squares to $I=I_{max} - \{I_{max} [Ant]^{nH}/(IC_{50}^{nH}+[Ant]^{nH})\}$, where the IC_{50} is the inhibition dose that blocks 50% of the current generated by 1 μM GABA and n_H is the hill coefficient. IC_{50} values are expressed as mean±S.E.M. (n=3-6). K_B values are the apparent dissociation constants for the antagonists, and were determined using Schild plot analysis (Arunlakshana and Schild, 1959). $-\log K_B$ values were determined using the following equation: $\log\{(A)/(A^*)-1\}=m.\log[Ant]-\log K_B$, where A is the EC_{50} of GABA in the presence of a known antagonist concentration, A^* is the EC_{50} of GABA in the absence of an antagonist, [Ant] is the concentration of the antagonist, and 'm' is the slope of the curve. For simple competitive antagonism, 'm' is 1. $-\log K_B$ values were determined by fitting data to the above function using Kaleidagraph 2.1 (1990). Schild analyses were carried out for compounds that had IC_{50} values of less than 30 μM.

Example 1 $GABA_C$ Receptor Antagonists Block Activation of Chloride Channels by GABA

Expression of human r_1 mRNA in *Xenopus* oocytes generated $GABA_C$ receptors which showed a dose-dependent GABA activated inward current when the cell was voltage clamped at -60 mV. This could be blocked by compounds such as CGP44530, CGP38593, CGP70523, CGP70522 and CGP36742, as shown in Figure 1. The structures of the compounds are shown in Figure 2 and Figure 3. These compounds were first screened at 100 μM to determine agonist activity, by activation of Cl^- channels, or antagonist activity, by blocking the activation of the channels by 1 μM GABA. Figure 2 shows the active compounds that had some effect at 100 μM as agonists (Figure 2A) or antagonists (Figure 2B) at $GABA_C$ receptors, and Figure 3 shows the compounds that

had no effect at 100 μM as agonists or antagonists at $GABA_C$ receptors.

Only the carboxylic acids, TACA, GABA and CACA activated the Cl^- channel. TACA was more potent than GABA, with an EC_{50} of 0.44 ± 0.02 μM , and was almost a full agonist, with a maximal TACA dose generating 95% of the maximal GABA activated current. GABA was found to have an EC_{50} value of 0.82 ± 0.09 μM. CACA was less potent than GABA, with an EC_{50} value of 37.4 ± 6.1 μM, and was a partial agonist, with a maximal CACA dose generating 75% of the maximal GABA activated current. These results are summarised in Table 1. The Hill Coefficients (n_H) as shown in Tables 1 and 2 were greater or equal to 2, which suggests that more than one molecule of the agonist is required to bind before the Cl^- channels can open. These findings are in agreement with those of Woodward *et al* (1993).

Table 1

Summary of EC_{50}, IC_{50}, K_B and Hill Coefficients of various agonists and antagonists at the $GABA_C$ Receptor Expressed in *Xenopus* oocytes.

	EC_{50} (μM)[a]	IC_{50} (μM)[b]	n_H[c]	K_B (μM)[d]
GABA	0.82±0.09		2.6±0.2	
TACA	0.44±0.02		2.4±0.2	
CACA	37.4±6.1		2.2±0.3	
Isoguvacine[e]	99			
CGP35024		0.75±0.07	1.8±0.1	0.58±0.14
CGP44530		5.5±1.2	2.4±0.5	8.6±1.6
CGP70523		38.9±4.9	1.6±0.1	
CGP27492		2.47±0.04	1.9±0.2	3.2±1.0
CGP38593		7.7±0.7	1.8±0.4	15.5±1.7
CGP70522		>100		
CGP36742		62.5±0.5	3.0±0.4	
TPMPA[e]				2.1

[a] EC_{50} is the effective dose that activates 50 % of the maximal current when tested at r_1 receptors expressed in *Xenopus* oocytes.

[b] IC_{50} is the concentration that inhibits 50% of the response produced by 1 μM GABA. Data are the mean±S.E.M. (n = 3-6 oocytes).

[c] n_H is the Hill Coefficient.

[d] K_B is the binding constant for the antagonist. These were determined using Schild plot analysis assuming competitive antagonism over the tested concentrations (Table 2).

[e] Data from Murata *et al*, 1996.

Table 2

Results of Schild Analyses of CGP35024, CGP27492, CGP44530 and CGP38593 at the $GABA_C$ Receptor Expressed in *Xenopus* oocytes.

Antagonist	[Antagonist] (μM)	EC_{50} (μM) of GABA[a]	n_H[b]	Slope of Schild Plot[c]
CGP35024	3	4.5±0.1	2.3±0.1	1.14
	10	10.0±1.4	2.2±0.2	
	30	28.8±4.2	1.9±0.2	
CGP27492	10	3.2±0.2	2.3±0.1	0.99
	30	9.3±1.4	2.4±0.3	
	100	25.7±0.1	2.5±0.2	
CGP44530	10	1.85±0.04	2.6±0.2	1.01
	30	3.2±0.2	3.0±0.1	
	100	10.7±0.5	3.7±0.4	
CGP38593	30	2.5±0.1	2.7±0.1	0.95
	60	4.1±0.2	2.5±0.4	
	100	6.8±0.3	3.0±0.1	

[a] EC_{50} is the effective dose that activates 50 % of the maximal current when tested at r_1 receptors expressed in *Xenopus* oocytes. EC_{50} values are expressed as mean±S.E.M. (n=3-6) and are determined by fitting data from individual oocytes using Kaleidagraph 2.1 (1990). EC_{50} values of GABA have shifted to the right in the presence of a known concentration of the antagonist. -log K_B values were determined as described in Materials and Methods section. The K_B values are shown in Table 1.

[b] n_H is the Hill Coefficient. These are greater than 1 indicating that more than 1 molecule of GABA is required for the channel to open.

[c] Slope of Schild plot analysis indicating competitive antagonism over the tested concentrations.

GP35024, CGP27492, CGP44530, CGP38593, CGP70523 and CGP70522 did not activate any current on their own (Figure 1). They acted as $GABA_C$ receptor antagonists, inhibiting the current activated by 1 µM GABA (Figure 1). IC_{50} values were obtained for these compounds (Table 1) and Schild analyses were carried out for the active compounds (Table 2). K_B (binding constant) values for CGP27492, CGP44530, CGP35024, and CGP38593 are shown in Table 1.

The methylphosphinic analogue, CGP44530, and phosphinic analogue, CGP38593 of TACA were antagonists with IC_{50} values of 5.5±1.2 µM and 7.7±0.7 µM respectively. These compounds had lower affinity for the $GABA_C$ receptor expressed in *Xenopus* oocytes than that of the corresponding methylphosphinic analogue, CGP35024, and phosphinic analogue, CGP27492, of GABA. CGP35024 had an IC_{50} of 0.75±0.07 µM and CGP27492 had an IC_{50} of 2.47±0.04 µM. The methylphosphinic analogue, CGP70523 and phosphinic analogue, CGP70522, of CACA were antagonists, with IC_{50} values of 38.9±4.9 µM and >100 µM respectively. These

compounds had lower affinity for $GABA_C$ receptors than the methylphosphinic and phosphinic analogues of GABA and TACA. The order of potency of the methyl phosphinic acids and phosphinic acids is CGP35024 > CGP27492 > CGP44530 > CGP38593 > CGP70523 >> CGP70522.

The new compounds CGP44530, CGP38593, CGP70523 and CGP70522 were weaker at the $GABA_C$ receptor than the existing phosphinic acid, CGP27492 and the methylphosphinic acid, CGP35024.

CGP35024, CGP27492, CGP44530 and CGP38593 were found to be competitive antagonists. The gradients of the Schild regression plots were not significantly different from 1 over the concentrations tested, indicating that these compounds compete for the same site as GABA.

CGP36742 was found to be an antagonist with moderate potency at the $GABA_C$ receptor, with an IC_{50} value of 62.5±0.5 μM. This compound is orally active, showing cognitive enhancement effects. Other related compounds, such as CGP35348, CGP46381, CGP51176 and CGP55845A (Figure 3), are also orally active, but do not show cognitive enhancement effects. These were screened at 100 μM, and had no effect as either agonists or antagonists at $GABA_C$ receptors. These compounds show high selectivity as $GABA_B$ receptor antagonists.

Example 2 Relative Effects of Compounds on $GABA_A$, $GABA_B$ and $GABA_C$ Receptors

The development of many alkylphosphinic and phosphinic analogues of GABA has yielded novel $GABA_B$ receptor agonists and antagonists (Olpe *et al*, 1990; 1993; Bittiger *et al*, 1992; 1993; Froestl *et al*, 1992; 1995a; 1995b), including the methylphosphinic and phosphinic analogues of TACA and CACA, *ie*. CGP44530, CGP38593, CGP70522 and CGP70523. In this study, we tested these compounds on $GABA_C$ receptors expressed in *Xenopus* oocytes, and found them to be competitive antagonists. The antagonist potencies of CGP44530, CGP38593, CGP70522 and

CGP70523 were found to be lower than that of the methylphosphinic and phosphinic analogues of GABA, CGP35024 and CGP27492.

The relative effects of the compounds at $GABA_A$, $GABA_B$ and $GABA_C$ receptors are shown in Table 3. Three compounds, CGP38593, CGP70522 and CGP27492, were moderately potent at $GABA_A$ receptors when tested using radioligand binding assays (IC_{50} = 6.8 µM; IC_{50} = 6.6 µM and IC_{50} = 1.7 µM, respectively) (Froestl *et al*, 1995a). However, the compounds were more potent at $GABA_B$ receptors than at $GABA_A$ receptors using this assay. Similarly, these compounds appear more potent at $GABA_B$ receptors than at $GABA_C$ receptors.

Table 3

Affinities of the compounds used in this study at GABA receptors.

	Receptor Affinity[a]		
Compound	$GABA_A$ (µM)[b]	$GABA_B$ (µM)[c]	$GABA_C$ (µM)[d]
GABA	0.128[k]	0.033	EC_{50} = 0.82[e]
CGP27492	1.7[k]	0.005	2.47
CGP35024	inactive at 10[k]	0.016	0.75
CGP36742	508	38	62
TACA	0.14[f, k]	inactive at 100[g]	EC_{50} = 0.44[e]
CGP38593	6.8	0.28	7.68
CGP44530	inactive at 100	0.65	5.53
CACA	25[f, k]	inactive at 100[g]	EC_{50} = 37[e]
CGP70522	6.6	4.4	> 300
CGP70523	242	16	38
Isoguvacine	1.4[f, k]	inactive at 500[h]	EC_{50} = 99[i]
TPMPA	K_b = 320[j]	EC_{50} ~ 500[h]	K_b = 2.1[i]
PMPA	> 100	> 1000[l]	6.0

[a] Receptor affinities are IC_{50} values unless otherwise stated.

[b] IC_{50} values *ie.* concentration that inhibits 50% of the total [^{3}H]muscimol binding using rat cortical membranes (Froestl *et al*, 1995a; 1995b).

[c] IC_{50} values for the inhibition of [^{3}H]CGP27492 binding using rat cortical membranes (Froestl *et al*, 1995a; 1995b).

[d] IC_{50} values for the inhibition of the response of 1 µM GABA using human r_1 mRNA expressed in *Xenopus* oocytes as described in the Materials and Methods section.

[e] EC_{50} values ie. the effective dose that activates 50 % of the maximal current when tested at r_1 receptors expressed in *Xenopus* oocytes as described herein.

[f] IC_{50} values for the inhibition of the total Na-independent [^{3}H]GABA binding using rat brain membranes (Johnston *et al*, 1978).

[g] Data from Kerr and Ong (1995) using guinea pig ileum, in the presence of bicuculline, against baclofen-depression of twitch contractions.

[h] Data from Ragozzino *et al* (1996) using whole cell patch recordings from pyrimidal neurons in hippocampal slices in the presence of bicuculline (20 µM).

[i] Data from Murata *et al* (1996) using human r_1 mRNA expressed in *Xenopus* oocytes.

[j] Data from Ragozzino *et al* (1996) using poly(A)$^+$ RNA from rat cortex expressed in *Xenopus* oocytes.

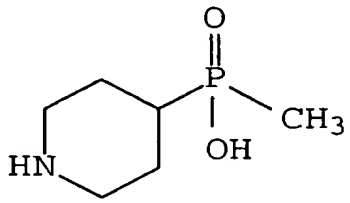
[k] EC_{50} values for GABA, CGP27492, CGP35024, TACA, CACA and isoguvacine using poly(A)$^+$ RNA from rat cortex expressed in *Xenopus* oocytes are 107 µM, 938 µM, inactive at 1 mM, 133 µM, inactive at 5 mM, and 305 µM respectively (Woodward *et al*, 1993). These values are different to the values obtained from radio-ligand binding assays.

[l] Data from measurement of the frequency of spontaneous discharges in rate neocortical slices using the grease-gap recording system.

Example 3 Specificity of $GABA_C$ Antagonists for $GABA_C$ Receptor Subtypes

We have demonstrated that the benchmark $GABA_C$ antagonist, TPMPA, is an order of magnitude less potent at blocking human homo-oligomeric rho-2 receptors than rho-1 $GABA_C$ receptors.

Of particular interest is the dihydro derivative of TPMPA, piperidine-4-(methyl)phosphinic acid (PMPA):



PMPA

(Piperid-4-yl)methylphosphinate (PMPA) was synthesized by reduction of a precursor of TMPA and subsequent hydrolysis, as follows:

Platinum oxide ($PtO_2.H_2O$) (50 mg) was added to a solution of recrystallised Troc-precursor (isopropyl [1-(2,2,2-trichloroethoxycarbonyl)-1,2,5,6-tetrahydropyridin-4-yl]methylphosphinate,A) (1.50 g, 3.96 mmol) in methanol (25 mL) and the mixture was shaken with H_2 (5 atm.) at room temperature for 24 h. The catalyst was filtered off through Celite, and the residue concentrated under reduced pressure to afford a viscous colourless oil. A n.m.r. examination of the crude reduction product indicated complete reduction of the olefinic bond together with significant concomitant reduction and partial deprotection of the Troc group.

A mixture of the residue from above, 48% aq. HBr (40 mL) and glacial *acetic* acid (40 mL) was refluxed for 60 h. The reaction mixture was concentrated under reduced pressure (water pump) and the final traces of HBr/AcOH were removed by the sequential addition of H_2O and concentration

(several cycles). The final residue (HBr salt) was dissolved in a small volume of H_2O and applied to a Dowex AG 50 (H^+) column. After initial elution with H_2O until the eluant was neutral, the eluting agent was changed to 1M aq. pyridine. Ninhydrin-positive fractions were combined and concentrated under reduced pressure (water pump). Final traces of pyridine were removed by the sequential addition of H_2O and concentration under reduced pressure (several cycles) to afford a quantitative yield of crude (piperid-4-yl)methylphosphinic acid (PMPA)(C) as an off-white solid (ca. 645 mg, air dried) which was recrystallised from EtOH/H_2O (450 mg, 70%): m. p. 289-291°; ^{1}H NMR (300 MHz, D_2O, Ref: DOH = δ 4.8) δ 1.19 (3H,d,J = 13.2 Hz, PCH_3), 1.56-1.82 (3H, 2 × overlapping m, 2 × NCH_2CH_B and PCH), 2.00-2.08 (2H, m, 2 × NCH_2CH_A), 2.96 (2H, (apparent?) dt, J = 3.0, 12.8 Hz, 2 × NCH_BCH_2), 3.43-3.51 (2H, m, 2 × NCH_ACH_2); ^{13}C NMR (D_2O, 75.64 MHz, Ref: (internal) dioxane = δ 67.4) δ 13.6 (d, J = 91.5 Hz), 23.1, 36.0 (d, J = 96 Hz), 44.8 (d, J = 14.2 Hz). The chemical synthesis is shown in Figure 5.

We have found that PMPA is a much more potent antagonist than TPMPA against rho-2 receptors, and less potent than TPMPA against rho-1 receptors, as indicated in Table 4.

Table 4

Binding Affinity for rho-1 and rho-2 Receptors

K_B (μM)	human rho-1 receptor	human rho-2 receptors
TPMPA	2.0 ± 0.4	15.6 ± 1.6
PMPA	6.0 ± 1.2	4.2 ± 0.2

PMPA and TPMPA show similar weak activity against $GABA_A$ and $GABA_B$ receptors.

The finding that TPMPA and PMPA show differing selectivity between rho-1 and rho-2 subtypes of $GABA_C$ receptors was quite unexpected.

Although the possibility that PMPA might have activity as a competitive antagonist of $GABA_C$ receptors is mentioned in U.S. Patent No. 5,627,169: "Selective Antagonists for $GABA_{rho}$ Receptor" and in a paper by Woodward *et al* (1993), it appears that neither this compound nor its analogues was actually synthesised and tested. Consequently these prior disclosures are merely speculative *paper examples*.

CGP36742 was shown to be a moderately potent antagonist at $GABA_B$ receptors using a $[^3H]$-CGP27492 binding assay (IC_{50} = 35 μM) (Bittiger *et al*, 1992; Olpe *et al*, 1993; Froestl *et al*, 1995a). It had weak effects at $GABA_A$ receptors (IC_{50} = 500 μM) (Bittiger *et al*, 1992), and had no effect at other receptor types, including NMDA, benzodiazepine, quisqualate, kainate, muscarinic cholinergic, adrenergic, serotoninergic and histaminergic receptors (1 mM) (Bittiger *et al*, 1992; Froestl *et al*, 1995b). However, we have now found that CGP36742 showed moderate antagonist activity at $GABA_C$ receptors (IC_{50} = 62 μM), and that its apparent selectivity for $GABA_B$ compared to $GABA_C$ receptors was approximately 2-fold. This compound has shown promising therapeutic potential in the treatment of cognitive deficits, petit mal epilepsy and depression (Bittiger *et al*, 1992). Therefore it is possible that antagonism of $GABA_C$ receptors contributes to the cognitive enhancement potentiation by CGP36742, such enhancement is not shown by other orally-active $GABA_B$ receptor antagonists (Froestl *et al*, 1995b).

TPMPA was recently synthesised and tested at $GABA_A$, $GABA_B$, and $GABA_C$ receptors (Murata *et al*, 1996). It is a conformationally-restricted analogue of CGP44530, and is the methylphosphinic analogue of isoguvacine. It was found to be more than 100-fold more selective as an antagonist for $GABA_C$ receptors than for $GABA_B$ receptors, and is 500-fold more selective at $GABA_C$ receptors than at $GABA_A$ receptors (Murata *et al*, 1996; Ragozzino *et al*, 1996).

Example 4 Effect of TPMPA on Memory

The effects of TPMPA in memory consolidation in chicks

This procedure trains each chick on anthranilate-coated red beads, which have a bitter taste. In the test, 120 min. after the initial exposure to the red bead each chick is presented with a blue and a red bead, and normally will avoid pecking at the red bead; the discrimination ratio measures how well it remembers to do this. Chicks trained on 100% anthranilate- coated beads produce a discrimination ratio better than 0.9, and drug- induced memory deficits can be detected in this group. However, chicks trained on 20% anthranilate -coated beads produce a discrimination ratio of around 0.6, and this group can be used to detect drug -induced memory enhancement. Drugs are delivered by two bilateral intracranial injections (10μL each).

Figure 6 shows that TPMPA at a dose of 30 μM enhances memory with the group trained with 20% anthranilate performing as well as the 100% anthranilate group. Figure 7 shows the dose- response relationship for the effects of TPMPA on discrimination ratio, with an EC_{50} between 1 and 10 μM. Figure 8 shows the dependence of this effect on time of injection, with an optimum effect produced by injecting in the 2.5 minutes after training.

The effects of TPMPA on the plus-maze memory test

In this assay mice are trained by placing them at the end of the open arm of the plus maze and allowing them to find the shelter of the closed arms. The time taken is measured as the 'latency'. Immediately after the trial the mice are injected with the test drug or with a saline control. Two and ten days later the test is repeated. An agent which enhances memory consolidation will significantly reduce the latency time relative to the

saline controls. The results are summarized in Figure 9, and show that TPMPA at 200 mg/Kg, but not at 50 mg/Kg, significantly reduces the latency in the 14 day test. This is consistent with enhancement of memory consolidation. When we repeated the experiment, this time testing only after 14 days and using Swiss mice from a different source, we found that TPMPA at 50 mg/Kg but not 200 mg/Kg significantly reduced latency. The experiments overall are therefore positive but inconclusive, since the different origin of the mice may be a contributing factor.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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CLAIMS

1. A method of enhancing cognitive activity in an animal, comprising the step of administering an effective amount of a compound which has $GABA_C$ receptor antagonist activity to an animal in need of such treatment.

2. A method of stimulating memory capacity in an animal, comprising the step of administering an effective amount of a compound which has $GABA_C$ receptor antagonist activity to an animal in need of such treatment.

3. A method according to claim 1 or claim 2, wherein the animal is suffering from a condition selected from the group consisting of cognitive deficit, memory impairment, and dementia.

4. A method according to any one of claims 1 to 3, wherein the animal is suffering from dementia, Alzheimer's disease, AIDS, or schizophrenia.

5. A method according to any one of claims 1 to 4, wherein the compound has selective antagonist activity against $GABA_C$ receptors compared with $GABA_B$ receptors.

6. A method according to any one of claims 1 to 5, wherein the compound has selective antagonist activity against $GABA_C$ receptors compared with $GABA_A$ receptors.

7. A method according to any one of claims 1 to 6, wherein the compound is substantially inactive against both $GABA_A$ and $GABA_B$ receptors.

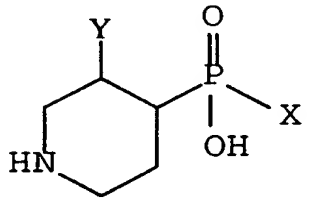
8. A method according to any one of claims 1 to 7, wherein the compound comprises a phosphinic acid group.

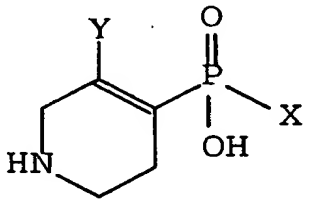
9. A method according to claim 8, wherein the phosphinic acid group is substituted with an alkyl group of 1 to 6 carbon atoms.

10. A method according to claim 7 or claim 8, wherein the compound comprises a double bond which imposes a conformational restriction on rotation about the bond corresponding to the C3-C4 bond of GABA.

11. A method according to any one of claims 1 to 10, wherein the compound is a conformationally-restricted analogue of CGP44530 in which rotation about the C3-C4 bond is restricted.

12. A method according to any one of claims 1 to 11, wherein the compound is represented by general formula I or general formula II:





(I) (II);

wherein in which X represents hydrogen, an alkyl group optionally substituted with a halogen, or a hydroxyalkyl group, and

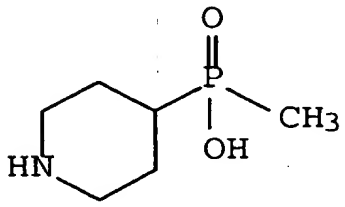
Y represents hydrogen, a halogen, or an alkyl, alkenyl, alkynyl or acyl group, optionally substituted with halogen, nitrile, or NO_2.

13. A method according to claim 12, wherein X is methyl or ethyl.

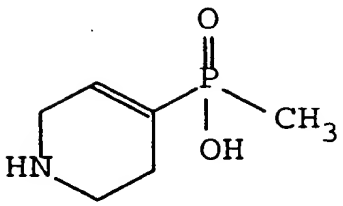
14. A method according to claim 12 or claim 13,

wherein the halogen is chlorine or fluorine.

15. A method according to any one of claims 12 to 14, wherein the compound is either:



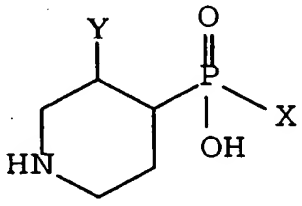
or



16. A method according to any one of claims 1 to 15, wherein the animal is a human.

17. A method according to any one claims 1 to 16, wherein the compound is administered orally.

18. A compound having $GABA_C$ antagonist activity and selectivity for the rho-2 subtype of $GABA_C$ receptors, of general formula I:



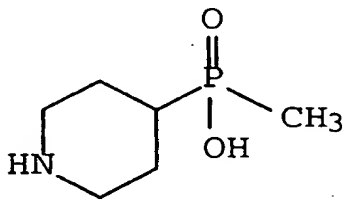
(I)

wherein X represents hydrogen, an alkyl group optionally substituted with a halogen, or a hydroxyalkyl group, and Y represents hydrogen, a halogen, or an alkyl, alkenyl, alkynyl, alkoxy or acyl group, optionally substituted with halogen, nitrile, or NO_2.

19. A compound according to claim 18, wherein X is methyl or ethyl.

20.. A compound according to claim 18 or claim 19, wherein the halogen is chlorine or fluorine.

21. A compound according to any one of claims 18 to 20, wherein the compound is:



22. A composition comprising a compound according to any one of claims 18 to 21, together with a pharmaceutically-acceptable carrier.

23. A method according to claim 1, substantially as hereinbefore described with reference to any one of the examples.

24. A compound according to claim 18, substantially as hereinbefore described with reference to any one of the examples.

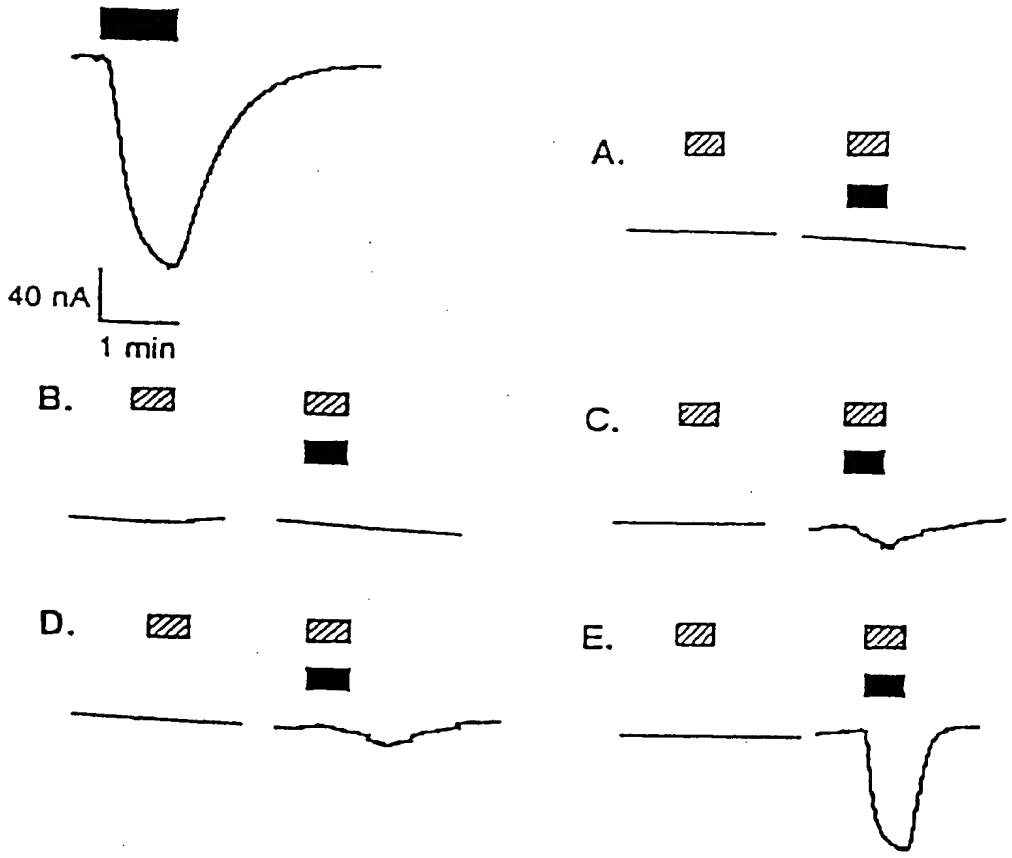
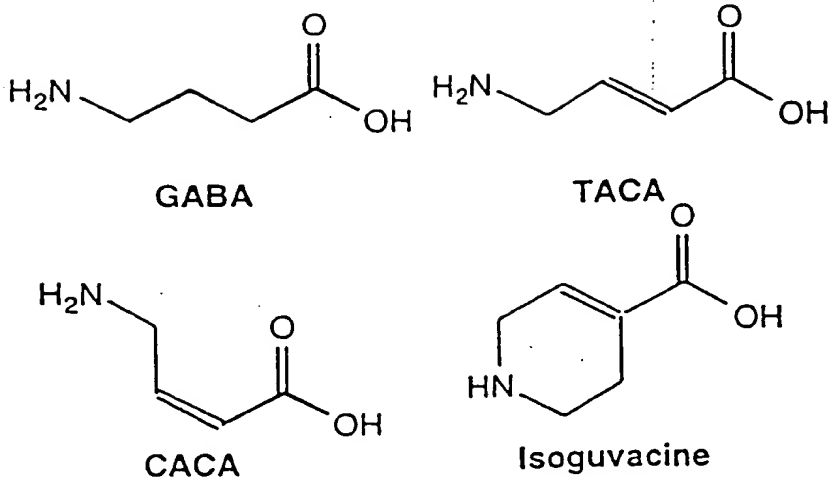


Figure 1

A.



B.

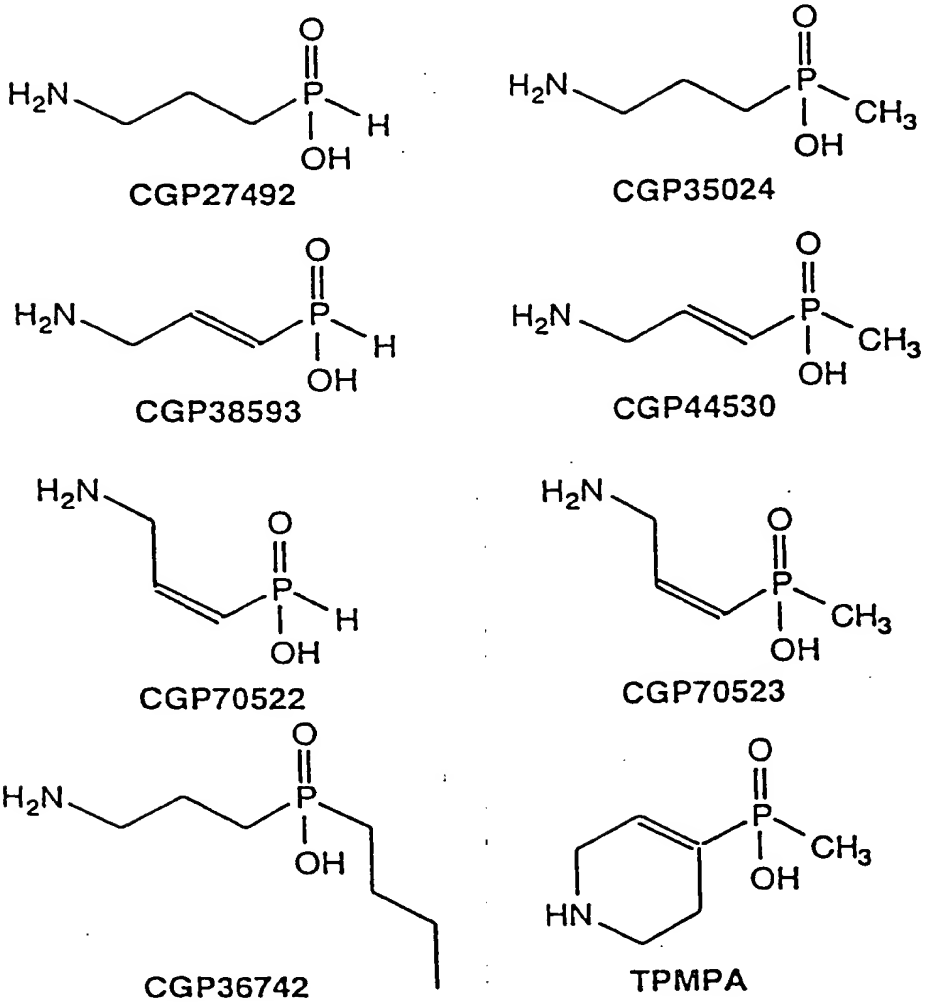
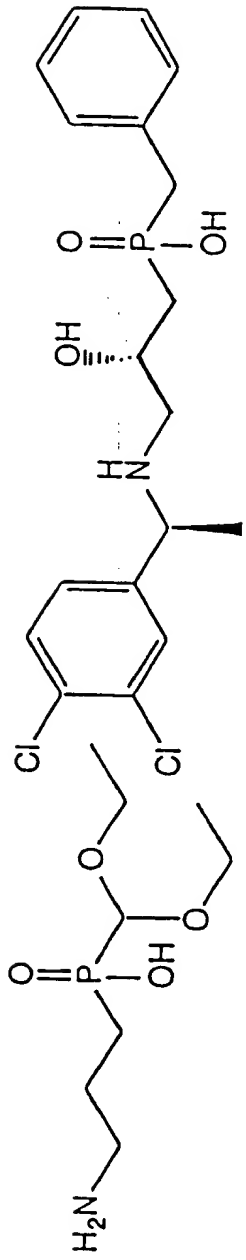
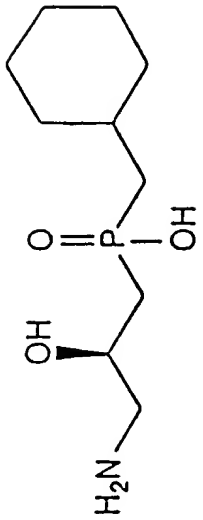


Figure 2





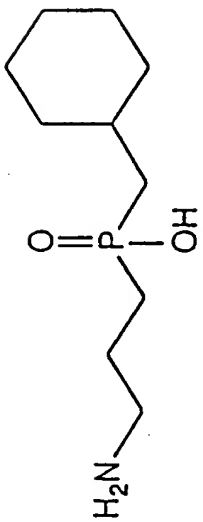


Figure 3

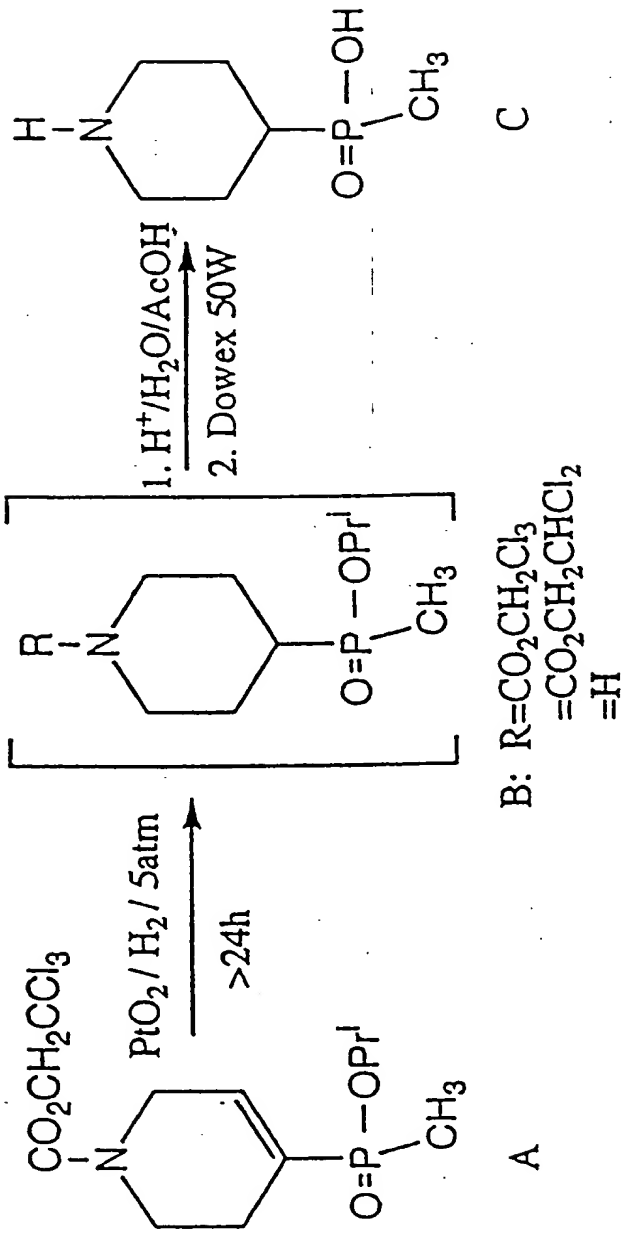

CO2CH2CCl3
PtO2 / H2 / 5atm
>24h
A
R
B: R=CO2CH2Cl3
=CO2CH2CHCl2
=H
1. H+/H2O/AcOH
2. Dowex 50W
H
C
OPri
CH3
O=P

FIGURE 4

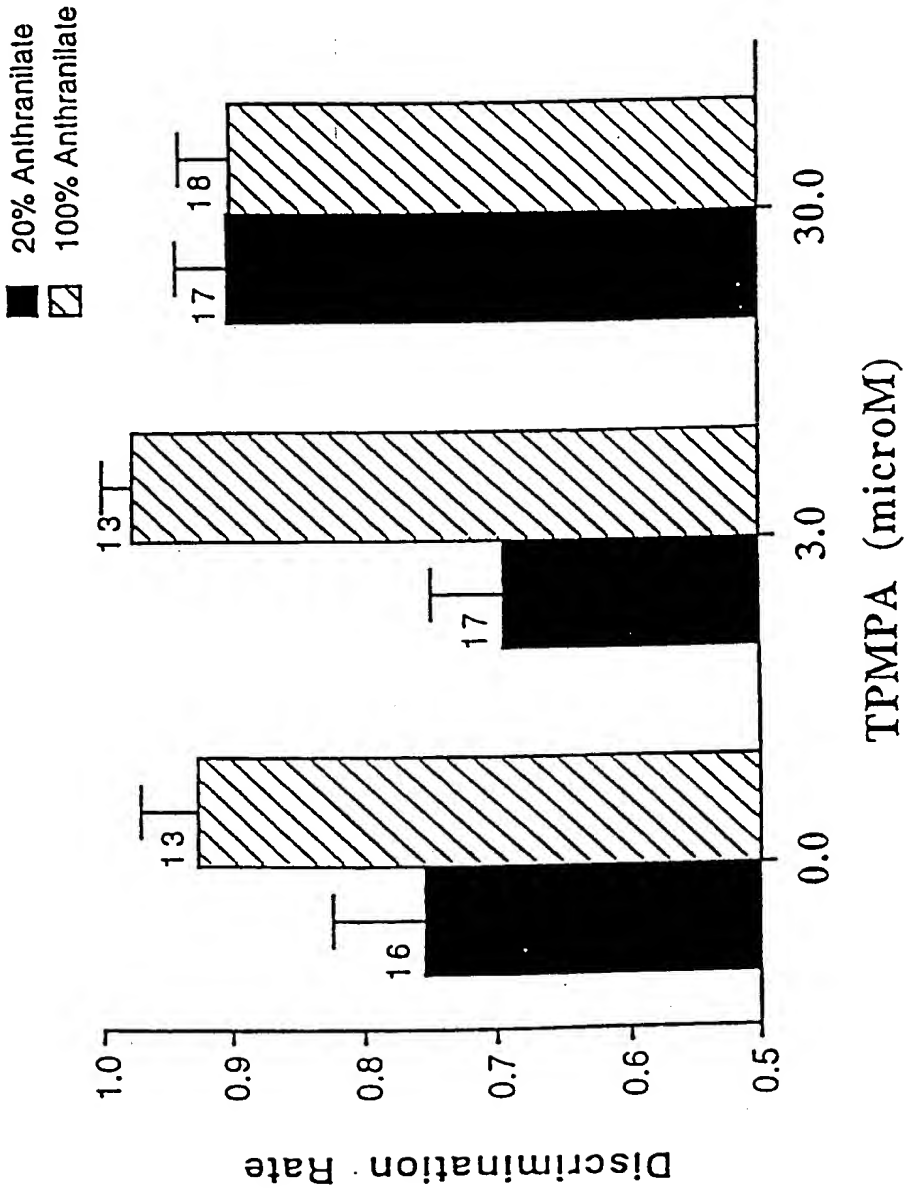

20% Anthranilate
100% Anthranilate
Discrimination Rate
1.0
0.9
0.8
0.7
0.6
0.5
16
13
17
13
17
18
0.0
3.0
30.0
TPMPA (microM)

FIGURE 5

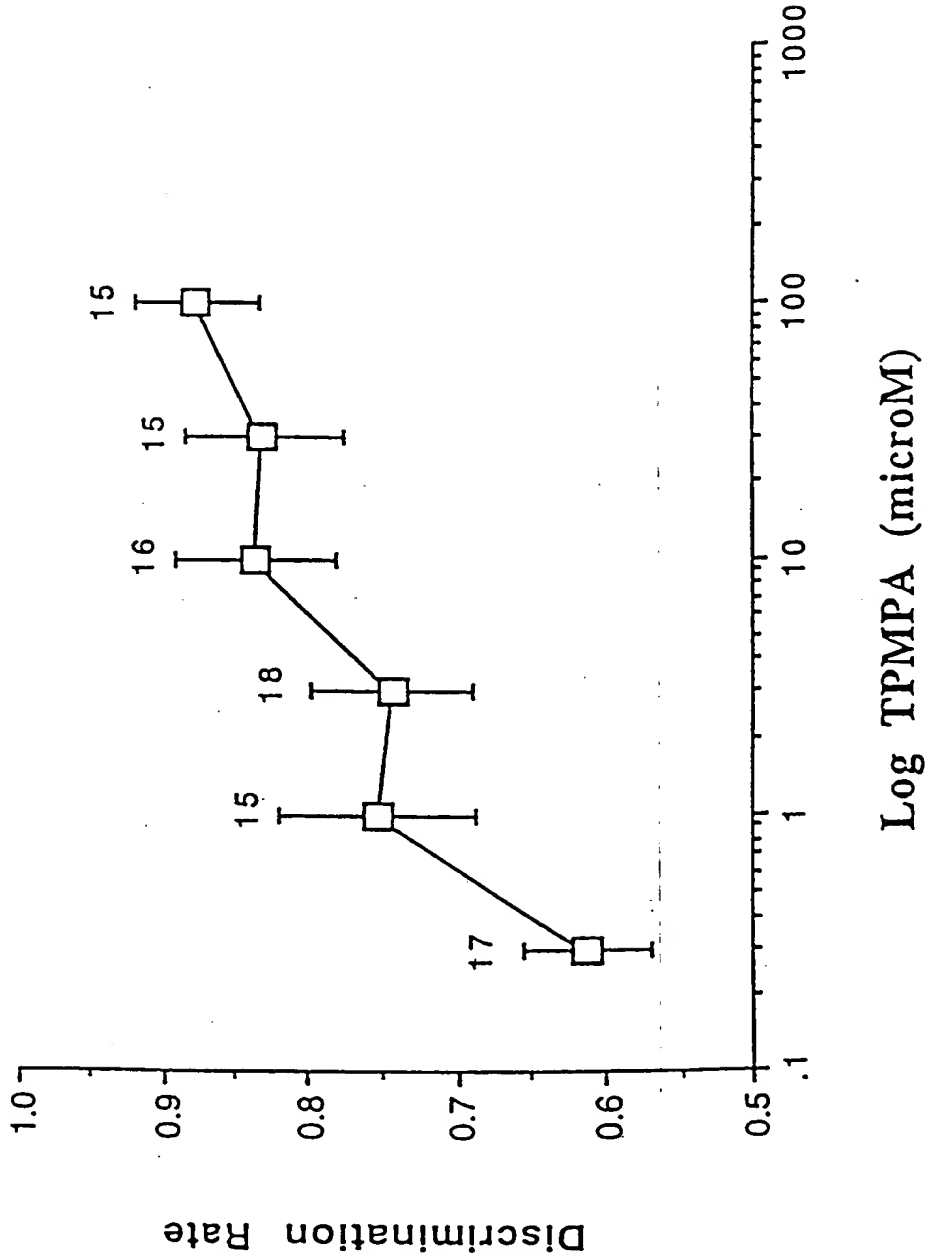

Discrimination Rate
1.0
0.9
0.8
0.7
0.6
0.5
.1
1
10
100
1000
Log TPMPA (microM)
15
15
16
18
15
17

FIGURE 6

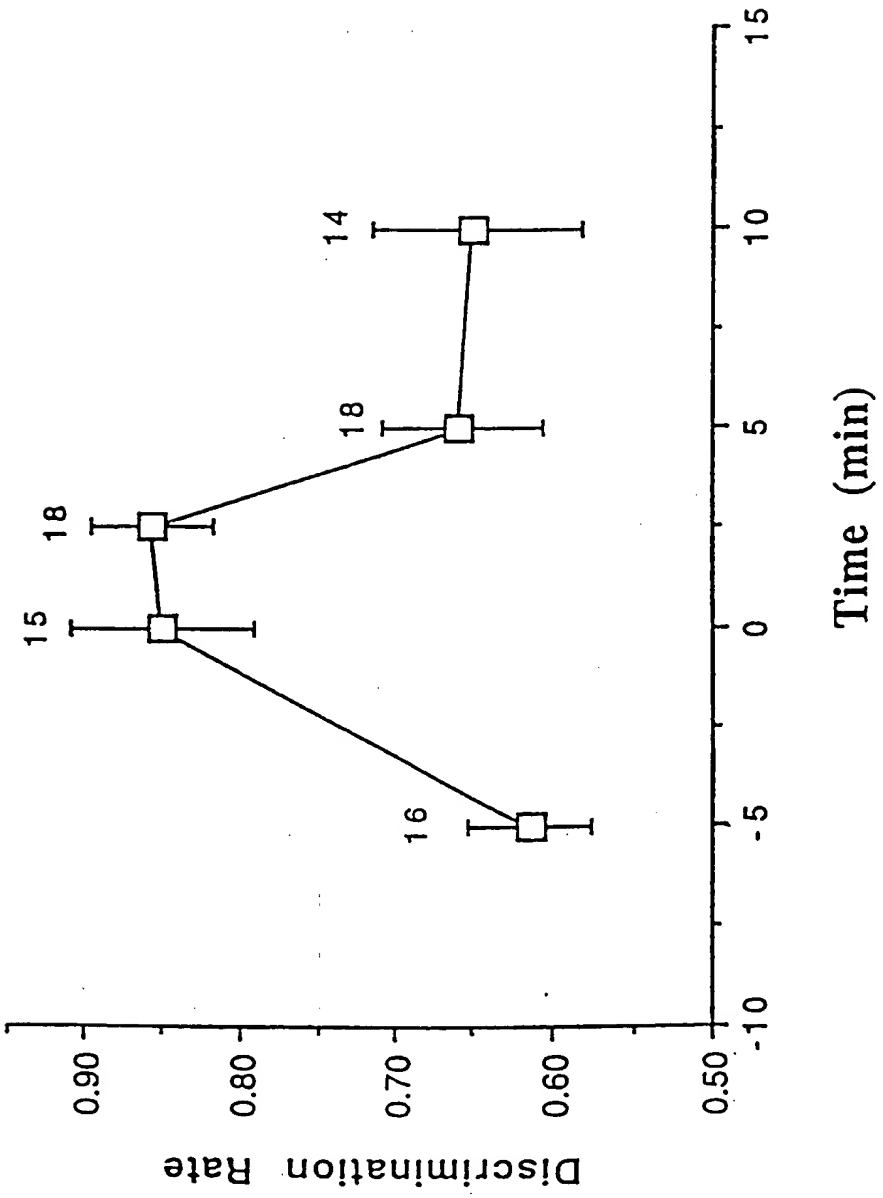

Discrimination Rate
0.90
0.80
0.70
0.60
0.50
Time (min)
15
10
5
0
-5
-10
14
18
18
15
16

FIGURE 7

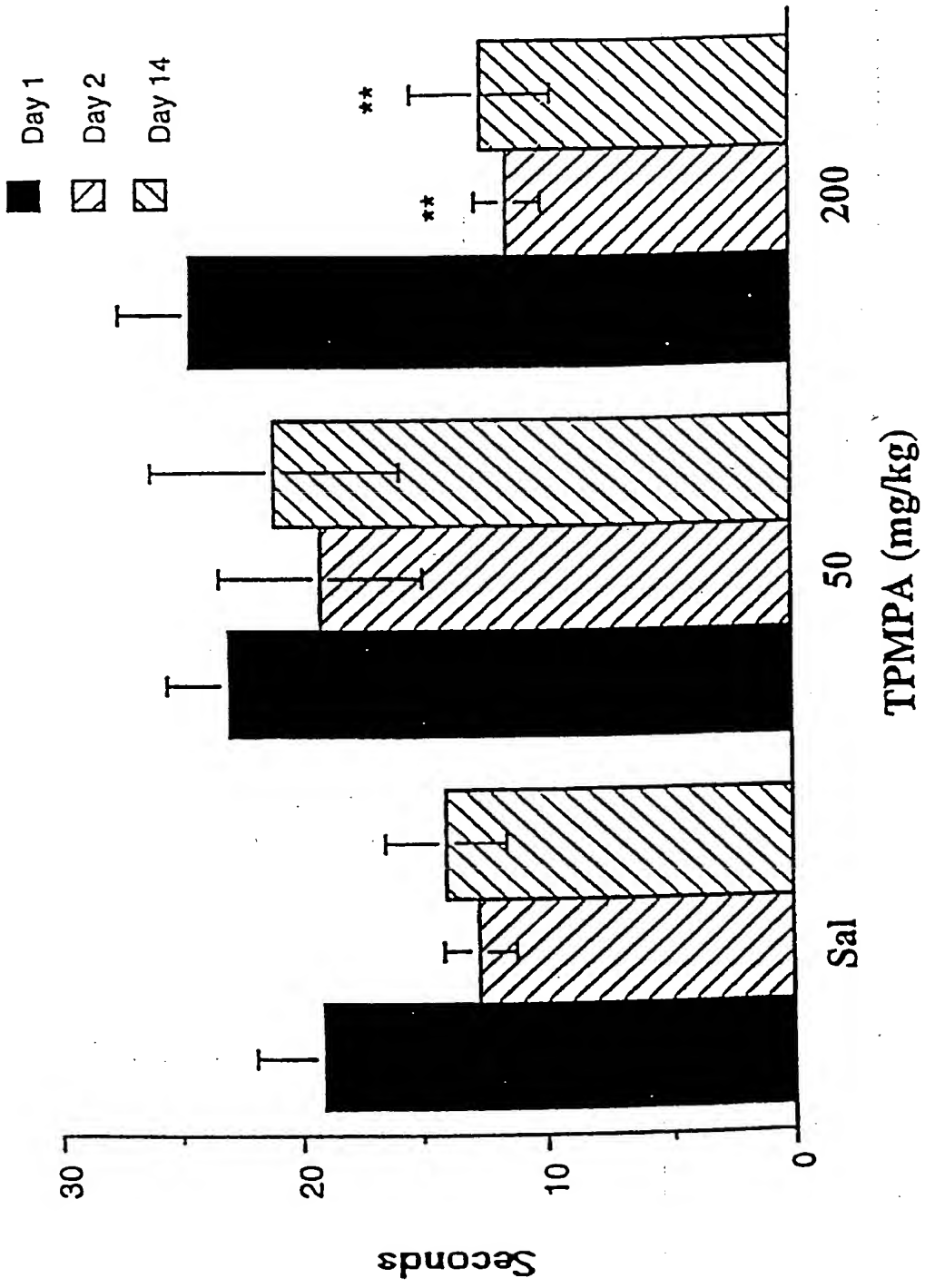


FIGURE 8

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00485

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07F 9/59; A61K 31/675;

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Derwent, Chem Abs: GABA C RECEPTOR: Gamma Aminobutyric acid receptor antagonist.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 5627169 (The Regents of The University of California.) 6 May 1997	18-22, 24 1, 3, 5, 6-9

☐ Further documents are listed in the continuation of Box C

☐ See patent family annex

* Special categories of cited documents:

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
	28 SEP 1998
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer A. WILCOX Telephone No.: (02) 6283 2243

Form PCT/ISA/210 (second sheet) (July 1992) copjhw